

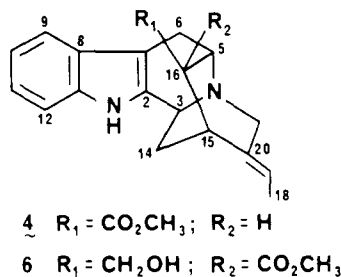
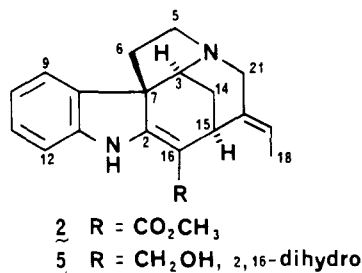
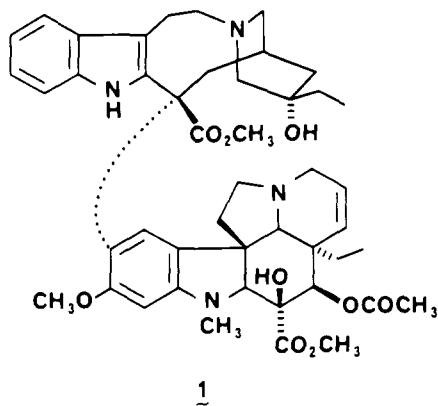
CATHARANTHUS ALKALOIDS. XXXVI.¹ ISOLATION OF VINCALEUKOBLASTINE (VLB) AND PERIFORMYLIN FROM *CATHARANTHUS TRICHOPHYLLUS* AND PERICYCLIVINE FROM *CATHARANTHUS ROSEUS*

SIBABRATA MUKHOPADHYAY and GEOFFREY A. CORDELL

Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, Illinois 60612

ABSTRACT.—The antineoplastic bisindole vincal leukoblastine (VLB) (1) and two monomers, akuammicine (2) and tetrahydroalstonine, have been isolated from *C. trichophyllus* roots, and periformylin (3) has been obtained from its leaf alkaloid fractions. Pericyclivine (4) and five alkaloids, leurosine, vincarodine, akuammicine, vindoline and ajmalicine, have been isolated from two acid fractions of *C. roseus* leaves. Carbon-13 nmr spectra of two monomeric indole alkaloids, akuammicine and pericyclivine, have been assigned.

As part of a continuing study of *Catharanthus* species for new biologically active substances, four alkaloids have been isolated from both the root and leaf alkaloid fractions of *Catharanthus trichophyllus* (Bak.) Pich. Prior phytochemical investigations in these laboratories have yielded eleven monomeric alkaloids along with a triterpene ursolic acid (2, 3) from this plant. In the present investigation, we report the first isolation of the important antineoplastic bisindole alkaloid



¹For Part XXXV, see Reference 1.

vincaleukoblastine (VLB) (1) from the root alkaloid fractions and a monomer, periformylne (3), from the leaf alkaloid fractions of *C. trichophyllus*.

The study of two acid fractions of *Catharanthus roseus* (L.) G. Don leaves has led to the isolation of six alkaloids, one of which, pericyclivine (4), has not previously been reported from this plant.

EXPERIMENTAL²

PLANT MATERIAL.—The *Catharanthus trichophyllus* (Bak.) Pich. (Apocynaceae) roots and leaves used in this study were obtained from the Medicinal and Poisonous Plant Garden of the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, and voucher specimens have been deposited in the herbarium of the above department.

PREPARATION OF ALKALOID FRACTIONS.—A sample of coarsely milled, air dried roots (1 kg) was extracted at room temperature for 24 hrs. by percolation with petroleum-ether (bp 40–80°). The extract was removed from the percolator and concentrated, and a fresh charge of solvent was added. The procedure was repeated four times. Pooling of the four concentrates was followed by evaporation *in vacuo* to afford fraction I (3.5 g).

The extracted *C. trichophyllus* roots were air dried and exhaustively extracted by repeated maceration and percolation with methanol. The combined percolates (ca. 5 liters) were concentrated to 250 ml and then extracted into 2 liters of a 2% tartaric acid solution. Residual methanol was removed *in vacuo*, and the acid solution was filtered. Five 250 ml volumes of ethyl acetate were used to extract the combined filtrates; the ethyl acetate extracts were pooled, dried (Na₂SO₄), filtered, and concentrated to give fraction II (2.7 g).

Residual ethyl acetate was removed from the aqueous acidic layer *in vacuo*, and the resulting solution was chilled and rendered alkaline with a saturated solution of sodium carbonate. The alkaline solution was then extracted with five 250 ml volumes of chloroform. The chloroform extracts, when pooled, washed with water until neutral, dried (Na₂SO₄), filtered, and evaporated *in vacuo*, yielded 2.6 g of crude tertiary bases (fraction III).

The above procedure was followed for the preparation of leaf fractions of *C. trichophyllus* (1 kg), and three corresponding fractions [fraction IV (20.0 g), fraction V (10.0 g) and fraction VI (1.5 g)] were obtained.

TABLE 1. Cytotoxicity of *Catharanthus trichophyllus* fractions.

Fraction	Test System	
	KB ED ₅₀ μg/ml ^a	P-388 ED ₅₀ μg/ml ^a
I.....	Inactive (100)	Inactive (100)
II.....	0.037	0.023
III.....	1.9	7.05
IV.....	Inactive (100)	Inactive (100)
V.....	0.28	0.14
VI.....	0.26	0.19

^aAn active fraction is one that exhibits an ED₅₀ < 20.0 μg/ml (4).

CYTOTOXICITY EVALUATION.—Fractions I–VI were evaluated for cytotoxicity against Eagle's 9KB carcinoma of nasopharynx and the P-388 lymphocytic leukemia test systems *in vitro* according to established protocols (4). The results are presented in table 1, and further phytochemical work-up was conducted on this basis.

²Melting points were determined by means of a Koffler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G grating spectrometer. The ir spectra were determined on a Perkin Elmer, model 710 spectrometer. Proton nmr spectra were recorded in CDCl₃ on a Varian model T-60A instrument with a Nicolet TT-7 Fourier Transform attachment and Carbon nmr spectra were recorded in CDCl₃ with a Varian XL-100 and Bruker HFX-90 instruments. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ-units (ppm). Optical rotations were measured with a Perkin-Elmer, model 241 polarimeter. High resolution mass spectra were obtained with an AEI MS 902 double focusing spectrometer operating at 70 ev.

CHARACTERIZATION OF VINCALEUKOBLASTINE (VLB) (1) AND AKUAMMICINE (2).—Fraction II was chromatographed over a column containing silica gel^s (100 gm) packed in chloroform. A total of 43 fractions (50 ml each) were collected from the column and combined on the basis of their tlc patterns. Fractions 18–22 from the column were combined and taken to dryness *in vacuo* (0.40 g) and the fraction was subjected to preparative tlc; the eluent used was methanol. Four bands were removed, and the band at R_f 0.35 was purified to afford a crystalline substance (5.8 mg, 0.0058%), mp 216°, $[\alpha]^{25D} + 42^\circ$ (CHCl₃); ν max (KBr) 3400 (s, NH and OH), 2950 (m), 1720 (s, ester CO), 1600 (m), 1460 (m), 1220 (s), 1120 (s), and 740 (m) cm⁻¹; uv, λ max (EtOH) (log ϵ) 217 (4.73), 265 (4.21) and 288 nm (sh); pmr δ (CDCl₃) 0.89 (t, $J=6$ Hz, 6H, 18-CH₃ and 18'-CH₃), 2.09 (s, 3H, 17-OCOCH₃), 2.70 (s, 3H, -NCH₃), 3.61 (s, 3H, 16'-CO₂CH₃), 3.79 (s, 6H, 16-CO₂CH₃ and 11-OCH₃), 5.27 (s, 1H, 17-H), 5.29 (d, $J=10$ Hz, 1H, 14-H), 5.78 (m, 1H, 15-H), 6.10 (s, 1H, 12-H), 6.64 (s, 1H, 9-H), and 7.64–7.14 (m, 5H, 9', 10', 11', 12'-H and indole NH). These physical data are in agreement with those reported for VLB (1) (5), and the identity was confirmed by comparison with an authentic sample.

Fractions 28–33 from the column were combined and taken to dryness *in vacuo* (0.19 gm) and further separated by preparative tlc; the eluant was ethyl acetate-abs. ethanol (3:1). The band at R_f 0.15 was removed and processed to afford a crystalline substance (5.4 mg, 0.0054%), mp 186°, $[\alpha]^{25D} - 72^\circ$ (c 0.5, EtOH); ir, ν max (KBr) 3340 (m, NH), 2920 (m), 1645 (s, CO), 1580 (s), 1450 (m), 1420 (m), 1290 (m), 1210 (m), 1080 (m), 1040 (m), and 730 (m) cm⁻¹; uv, λ max (MeOH) (log ϵ) 228 (4.09), 298 (4.07) and 328 nm (4.24); pmr, δ (CDCl₃) 1.60 (d, $J=6.8$ Hz, 3H, 18-CH₃), 3.79 (s, 3H, CO₂CH₃), 5.33 (m, 1H, 19-H) and 7.27–6.75 (m, 4H, 9, 10, 11, 12-H); cmr, see table 2; ms, m/e , M⁺ 322(31), 307(4), 293(2), 291(5), 279(3), 275(3), 263(11), 252(8), 247(5), 238(3), 234(8), 233(4), 225(4), 221(5), 218(6), 207(8), 206(15), 204(8), 193(11), 180(12), 1167(14), 166(7), 165(7), 154(8), 121(51), 119(10), 111(12), 109(12), 106(11), and 97(24).

These physical data are in agreement with those reported for akuammicine (2) (6), and the identity was confirmed (mmp, tlc and superimposable ir) by comparison with an authentic specimen.

CHARACTERIZATION OF PERIFORMYLIN (3).—Fraction VI was chromatographed over a column containing silica gel (100 gm) packed in chloroform. A total of 30 fractions were collected from the column and combined on the basis of their tlc patterns. Fractions 8–10, when evaporated from acetone afforded a solid (5.6 mg, 0.0056%), mp 208°, which exhibited the following spectral properties, ir, λ max (KBr) 3200 (m, NH), 2950 (m), 1735 (s, CO), 1660 (s, -NCHO), 1610 (s), 1420 (m), 1410 (s), 1340 (m), 1300 (m), 1210 (s), 1140 (m), 940 (m), and 740 (m) cm⁻¹; uv λ max (MeOH) (log ϵ) 242 (4.22) and 318 nm (4.32); pmr, δ (CDCl₃) 1.73 (d, $J=7.3$ Hz, 3H, 18-CH₃), 2.60 and 2.70 (s, 3H, CO₂CH₃), 5.62 (m, 1H, 19-H), 8.16 and 8.30 (s, 1H, NCHO), 9.08 (bs, 1H, NH, D₂O exchange); ms, m/e , M⁺ 366 (15%), 289(6), 194(9), 180(7), 173(12), 172(82), 166(9), 154(9), 144(9), 130(45), 129(28), 128(19), 108(20), 106(19), 102(24), 85(18) and 83(31). These physical data are in agreement with those reported for periformylin (3) (7), and the identity was confirmed by comparison with an authentic sample.

PREPARATION OF ALKALOID FRACTIONS OF *C. roseus*.—The source, identification, and processing of the leaves of *Catharanthus roseus* have been discussed previously (8).

SEPARATION OF THE CRUDE ALKALOID FRACTIONS PRE VLB CUT-2 (F-006) AND POST VLB CUT-11-12 (F-008).—Aliquots of the fractions F-006 and F-008 (10.0 gm each) were separately dissolved in chloroform and were placed at the top of two glass chromatographic columns containing chloroform slurries of silica gel PF 254 (100 gm each). Elution was accomplished initially with chloroform and then with solvents of increased polarity by the addition of methanol. Each fraction was reduced to dryness *in vacuo*, dissolved in 2 ml of eluting solvent, and monitored by tlc with ethyl acetate-abs. ethanol (3:1) and methanol as eluents with ceric ammonium sulphate as detecting reagent. Grouping of the fractions was accomplished on the basis of similar tlc patterns as well as chromogenic reactions of resolved alkaloids to the CAS detecting reagent. The fractions were monitored throughout by concomitant bioassay.

Ajmalicine (0.00002%), vindoline (0.00002%), akuammicine (0.000006%), vincarodine (0.000006%), and leurosine (0.000004%) have been isolated from different fractions and were characterized by uv, ir, pmr, ms and, also, comparison with authentic samples.

CHARACTERIZATION OF PERICYCLIVINE (4).—Repeated chromatography of the fraction F-006 over silica gel afforded a solid, which was crystallized from acetone (25.2 mg, 0.00002%) and exhibited the following properties, mp 228°, $[\alpha]^{25D} + 5^\circ$ (c 0.5, CHCl₃); ir, λ max (KBr) 3100 (m), 3020 (m), 2850 (m), 1735 (s), 1730 (s), 1420 (m), 1275 (m), 1190 (s), 1030 (s), 985 (m), 815 (m), and 700 (s) cm⁻¹; uv, λ max (EtOH) (log ϵ) 225 (4.51), 280 (3.88) and 289 sh nm; pmr, δ (CDCl₃) 1.60 (d, $J=6.7$ Hz, 3H, 18-CH₃), 3.06 (s, 3H, CO₂CH₃), 5.26 (q, $J=6.9$ Hz, 1H, 19-H), 7.47–7.0 (m, 4H, 9, 10, 11, 12-H), 8.0 (bs, 1H, NH, exchanged with D₂O); cmr, see table 2; ms, m/e , M⁺ 322(100), 321(90), 307(28), 263(39), 249(26), 235(9), 182(14), 181(13), 170(13), 169(97), 168(92), 167(12), 156(11), 154(15), and 115(13). These physical data are in accord with those published for pericyclivine (4) (9).

^sE. Merck, Darmstadt, W. Germany.

DISCUSSION

The cmr spectral data of two alkaloids, akuammicine (2) and pericyclivine (4), have been described in table 2. The assignments are based on the splitting pattern in the coupled spectra and also on comparison of the chemical shifts with those of model compounds. The aromatic carbon resonances in akuammicine (2) were in accord with those reported for the retuline-like substance (5) by Wenkert *et al.* (10, 17). The cmr spectrum of pericyclivine (4), however, was assigned by the use of off-resonance ^1H decoupled spectral information and correlation of carbon resonance frequencies with those of akuammidine (6) (11).

TABLE 2. Carbon-13 nuclear magnetic resonance spectra of akuammicine (2) and pericyclivine (4).

Carbon number	Akuammicine (2) ^a	Pericyclivine (4) ^a
2.....	167.73	139.37
3.....	61.74	43.77
5.....	56.13	55.91
6.....	46.15	26.80 ^b
7.....	57.42	105.47
8.....	136.80	127.03
9.....	120.26	119.17 ^c
10.....	120.26	117.40 ^c
11.....	127.40	121.25
12.....	109.10	110.86
13.....	143.20	137.48
14.....	29.59	24.15
15.....	30.77	27.21 ^b
16.....	100.95	50.26
17.....	167.73	172.94
18.....	12.65	12.85
19.....	120.53	114.36
20.....	139.10	136.64
21.....	56.77	52.99
CO ₂ CH ₃ ...	50.79	50.78

^aIn parts per million from TMS $\delta(\text{CDCl}_3) + 76.9$ ppm.

^{b,c}Assignments may be reversed.

The isolation of vincaleukoblastine (VLB) (1) was reported previously from *C. roseus* (5). Akuammicine (2), an alkaloid originally isolated from *Picalima nitida* (6, 12), has also been obtained from *Vinca erecta* (13), *V. minor* (14), *C. roseus* (15), and *C. trichophyllus* (3). Periformyline (3) was isolated previously only from *C. lanceus* (7), and pericyclivine (4) has been obtained from *C. lanceus* (16) and *Gabunia odoratissima* (9).

BIOLOGICAL ACTIVITY OF THE ISOLATES.—Tetrahydroalstonine, vincaleukoblastine (VLB), akuammicine, periformyline, ajmalicine, vindoline, vincarodine, leurosine and pericyclivine were examined for their activity against Eagle's 9KB carcinoma of the nasopharynx in cell culture and P-388 lymphocytic leukemia test system *in vitro* by established protocols (4). Vincaleukoblastine, leurosine and akuammicine (KB:ED₅₀ 2.4 and PS:ED₅₀ 1.7 $\mu\text{g}/\text{ml}$) were found to be active.

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